



REVIEW

Neutrophil recruitment and intracellular vesicle transport: A short overview

Sergi Masgrau-Alsina | Markus Sperandio  | Ina Rohwedder

Institute of Cardiovascular Physiology and Pathophysiology, Ludwig-Maximilians-University Munich, Planegg-Martinsried, Germany

Correspondence

Ina Rohwedder, Institute of Cardiovascular Physiology and Pathophysiology, Ludwig-Maximilians-University Munich, Planegg-Martinsried, Germany.
Email: ina.rohwedder@med.uni-muenchen.de

Markus Sperandio, Walter-Brendel-Zentrum für Experimentelle Medizin, LMU München, Großhadener Str. 9, 82152 Planegg-Martinsried, Germany.
Email: markus.sperandio@lmu.de

Funding information

Deutsche Forschungsgemeinschaft, Grant/Award Number: SFB914 and SP621/5-1; Else Kröner-Fresenius-Foundation, Grant/Award Number: 2015_A68

Abstract

Recruitment of neutrophils from the intravascular compartment into injured tissue is an essential component of the inflammatory response. It involves intracellular trafficking of vesicles within neutrophils and endothelial cells, both containing numerous proteins that have to be distributed in a tightly controlled and precise spatiotemporal fashion during the recruitment process. Rab proteins, a family of small GTPases, together with their effectors, are the key players in guiding and regulating the intracellular vesicle trafficking machinery during neutrophil recruitment. This review will provide a short overview on this process and highlight new findings as well as current controversies in the field.

KEYWORDS

inflammation, neutrophil, rab proteins, vesicle trafficking

1 | INTRODUCTION

For decades, haematologists have applied histology to distinguish white blood cell populations from each other. Hence, the first classifications of immune cells were based solely on morphological criteria. Using these criteria, neutrophils, together with eosinophils, basophils and mast cells were classified as granulocytes, reflecting the high density of vesicles in their cytoplasm. Later on, the appearance of neutrophil granules has been investigated during neutrophil maturation in the bone marrow and four granule classes could be defined based on their cargo. Azurophilic or primary granules, which appear in myeloblasts and promyelocytes; specific or secondary

granules, primarily found in myelocytes and metamyelocytes; gelatinase or tertiary granules, first produced in band neutrophils; and finally secretory vesicles, which are only found in segmented neutrophils.¹ Recently, a new type of granule, rich in ficolin-1 has been identified in myelocytes, metamyelocytes and band cells.²

The high density of granules in neutrophils points towards an important functional role during the acute inflammatory response, for example against invading pathogens. Indeed, degranulation is a hallmark of neutrophil activation and the different types of granules are released in a sequential order. Secretory vesicles, containing mainly adhesion molecules, are the first granules to be discharged,

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *European Journal of Clinical Investigation* published by John Wiley & Sons Ltd on behalf of Stichting European Society for Clinical Investigation Journal Foundation

followed by specific and gelatinase granules that allow cell migration and create a hostile environment. Finally, the high antimicrobial content of azurophilic granules is released at the site of injury³ (see Table 1 for further details). Proper vesicle release is imperative for neutrophil recruitment and requires the tightly controlled transport of vesicles from intracellular storage pools to the neutrophil surface. In this review, we will describe the molecular mechanisms of intracellular vesicle transport during neutrophil recruitment and highlight the role of Rab molecules in this process.

2 | VESICULAR TRAFFICKING MACHINERY

Intracellular vesicle transport is essential for maintaining cell homeostasis and proper cell function. This becomes evident by the wide range of pathologies, from neurodegenerative to autoimmune diseases, emerging from alterations of the vesicular machinery. Also, neutrophil function heavily relies on the ability to produce and release granule content. Depending on the vesicle directionality, protein transport can be classified into the exocytic/biosynthetic route and the endocytic/recycling route. Either way, the central gear in vesicular trafficking is constituted by a family of small GTPases called Rab proteins. Already present in the last eukaryotic ancestor, Rab proteins have been indispensable during the evolution of the endomembrane system and have shown a dramatic plasticity, correlating

with multicellularity and the increasing complexity in cell organization and specificity. So far, approximately 70 members of the Rab family have been identified in humans.⁴ Each Rab protein specifically coordinates the transport, tethering and fusion of a vesicle to its target compartment by interacting with several effector proteins, such as guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs) or tethering factors. Interaction of Rab proteins with their effectors contributes to define membrane domains that confer specificity in membrane trafficking. These membrane domains have been extensively studied in the endocytic pathway, where early endosomes (EE) are found to harbour only Rab5, late endosomes (LE) only Rab7, and Rab11 mainly in recycling endosomes (RE).⁵

Rab27 mostly controls the exocytic pathway. The isoform Rab27a plays an important role in neutrophil degranulation. When interacting with its effector JFC1, it mediates the release of azurophilic granule content, while when interacting with the docking factor Munc13-4, Rab27a mediates the release of azurophilic, secretory and gelatinase granules.¹ Its other isoform, Rab27b, has also been implicated in neutrophil secretion since its absence attenuates, but does not abolish, azurophilic granule exocytosis.^{6,7}

The machinery regulating endo- and exocytosis plays an essential role in the whole process of neutrophil recruitment, since the supply and spatial distribution of adhesion relevant proteins to the cell surface needs to be controlled in a very precise manner in both the neutrophil and the endothelial cell.

TABLE 1 Content of neutrophil granules. List of proteins that are found in the various granule classes

Azurophilic granules	Specific granules	Gelatinase granules	Secretory vesicles
CD63	CD11b/CD18	CD11b/CD18	CD10
CD68	CD66	NOX2	CD11b/CD18
MPO	CD67	MMP-9	CD15
NE	CD177	MMP-25	CD16
Cathepsin G	NOX2	fMLF-R	CD35
Proteinase 3	MMP-8	SCAMP	NOX2
NSP4	SCAMP	VAMP2	MMP-25
Cathepsin C	AI-at	Lysozyme	NRAMP2
α 1-AT	Lysozyme	Arginase I	SCAMP
Lysozyme	β ₂ -microglobulin Collagenase	Gelatinase	VAMP2
	Heptoglobin	Ficolin I	α 1-AT
	hCAP-18		Plasma proteins
	Lactoferrin		
	NGAL		
	Pentraxin 3		
	SLPI		

Note: Modified from Cowland and Borregaard 2018.³

Abbreviations: fMLF-R: *N*-Formylmethionyl-leucyl-phenylalanine; hCAP-18: human cathelicidin 18; NGAL: neutrophil gelatinase-associated lipocalin; NOX2: NAPDH oxidase 2; NRAMP2: natural resistance-associated macrophage protein 2; NSP4: non-structural glycoprotein 4; SCAMP: secretory carrier-associated membrane protein; SLPI: secretory leukocyte protease inhibitor; VAMP2: vesicle-associated membrane protein 2; α 1-AT: alpha1-antitrypsin.

3 | NEUTROPHIL RECRUITMENT CASCADE

Neutrophils circulate through the blood stream in a rather quiescent state, but after an insult occurs they become activated and are recruited to the sites of inflammation in order to re-establish homeostasis. Cell damage or invasion of microorganisms is detected by tissue-resident cells, which release inflammatory mediators that activate the surrounding environment including endothelial cells. This leads to neutrophil capture from free-flowing blood followed by rolling and firm adhesion of the neutrophil to the endothelium (Figure 1). Thereafter, neutrophils transmigrate through the endothelial layer and then penetrate the basement membrane before reaching the inflamed tissue. It is paramount that all the molecules involved in this process (some of them are contained in neutrophil secretory

vesicles), act synergistically in order to resolve the damage or infection.

The activation of the endothelium results in the surface mobilization of various adhesion molecules, either already present in storage granules or generated through de-novo synthesis. For example, the rolling receptor P-selectin is stored in Weibel-Palade bodies (WPB) of endothelial cells and rapidly mobilized to the luminal surface where it supports neutrophil capture and rolling through binding to neutrophil expressed P-selectin glycoprotein ligand-1 (PSGL-1).⁸ E-selectin is another member of the selectin family. In contrast to P-selectin, E-selectin has to be de-novo synthesized after stimulation of endothelial cells before it can be mobilized to the cell surface. Signalling through PSGL-1 stimulates activation of the two β_2 integrins, lymphocyte function-associated antigen-1 (LFA-1 or $\alpha_L\beta_2$) and macrophage antigen-1 (Mac-1 or $\alpha_M\beta_2$). These β_2 integrins establish strong bonds with intracellular adhesion

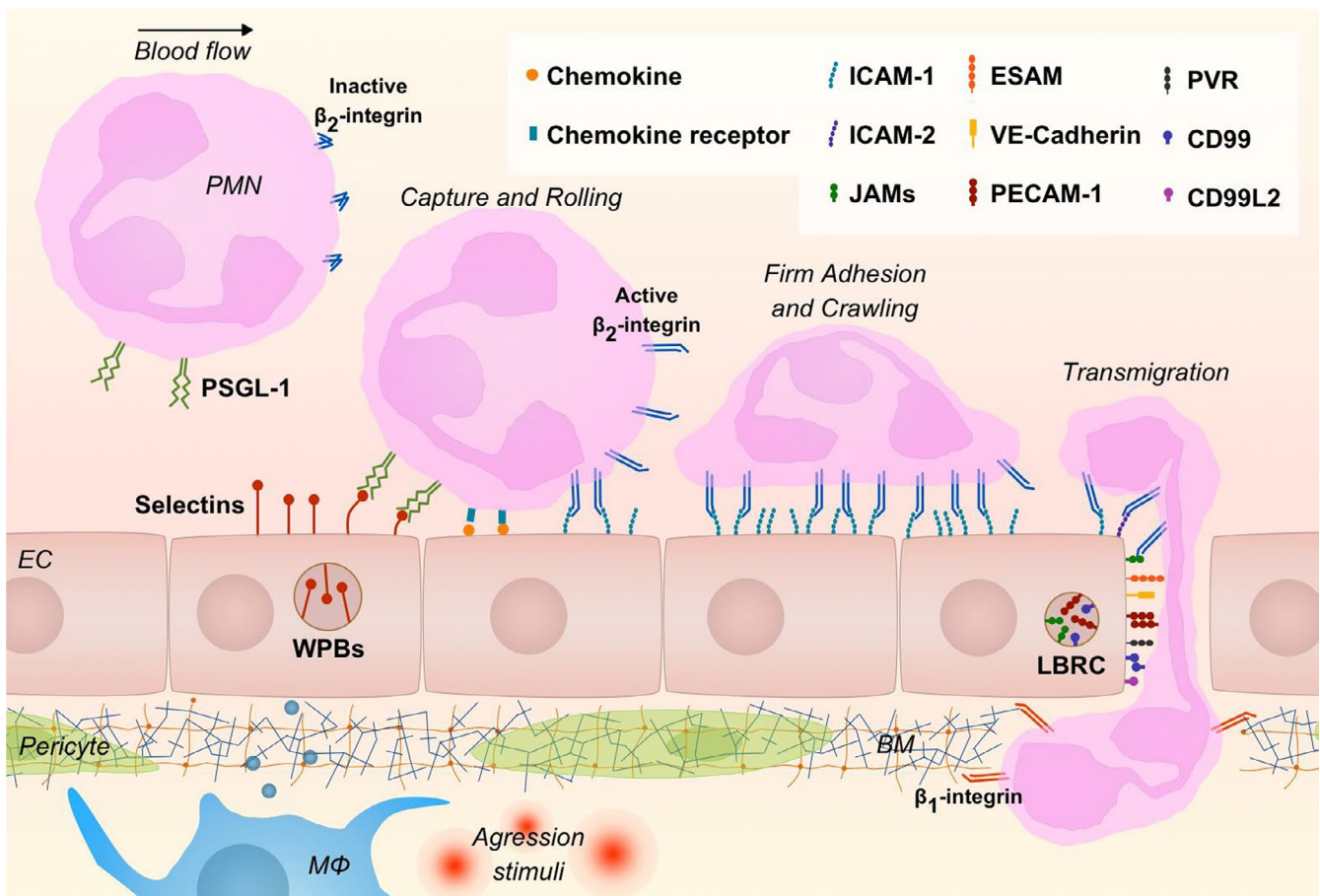


FIGURE 1 Leucocyte recruitment cascade. After sensing “danger” signals, sentinel cells within the tissue release mediators that stimulate endothelial cells and promote selectin expression on the endothelial cell surface. Neutrophil expressed PSGL-1 binding to endothelial selectins promotes neutrophil capture and rolling on the inflamed endothelium. PSGL-1-selectin interactions together with chemokine-induced signalling events promote β_2 integrin unfolding and activation. Binding of the β_2 integrin LFA-1 to ICAM-1 leads to neutrophil slow rolling and firm arrest. Thereafter, neutrophils start crawling to an appropriate site for extravasation, which usually occurs through a paracellular route involving the sequential use of ICAM-1, ICAM-2, JAMs, ESAM, PECAM-1, PVR, CD99 and CD99L2. Finally, neutrophils penetrate the vascular basement membrane to reach the inflamed tissue, a process depending on laminin-binding integrins VLA3 and VLA6 as well as neutrophil elastase. EC; endothelial cell; LBRC, lateral border recycling compartment; M Φ , macrophage; PMN, polymorphonuclear cell; WPBs, Weibel-Palade bodies

molecule 1 (ICAM-1), constitutively expressed on endothelial cells, leading to firm adhesion of neutrophils. This is followed by β_2 integrin clustering accompanied by postarrest modifications including neutrophil spreading and crawling.⁹ ICAM-1 dependent signalling stimulates Ca^{2+} flux in endothelial cells that leads to actin cytoskeleton rearrangement and facilitates leucocyte transendothelial migration (TEM). Several endothelial cell membrane proteins, such as intracellular adhesion molecule-2 (ICAM-2), junctional adhesion molecules (JAMs), endothelial cell-selective adhesion molecule (ESAM), platelet endothelial cell adhesion molecule-1 (PECAM-1), poliovirus receptor (PVR), CD99 and CD99L2, support transendothelial migration in a sequential fashion.¹⁰ Finally, neutrophils need to cross the underlying basement membrane before entering the interstitial space, a process that has gained a lot of interest recently and seems to be dependent on additional integrins such as very late antigen-3 (VLA-3 or $\alpha_3\beta_1$) and very late antigen-6 (VLA-6 or $\alpha_6\beta_1$), as well as neutrophil elastase (NE).^{11,12}

4 | NEUTROPHIL VESICLE TRAFFICKING

4.1 | Adhesion

During adhesion to the inflamed endothelium, neutrophils change their shape, flatten and convert from a non-polarized into a polarized cell. After stimulation with chemoattractants, the transmembrane kinase phosphatidylinositol 4-phosphate 5-kinase type-1 gamma (PIP5K1C-90) induces polarization by regulating Ras homolog family member A (RhoA) GTPase and local integrin activation.¹³ The Rab family member Rab21 has been described to carry vesicles containing PIP5K1C-90 to critical polarization sites through its effector rabphilin-3A (RPH3A), which increases its binding to Rab21 after being phosphorylated by kinase protein kinase N1 (PKN1) (Figure 2A).¹⁴

The strong interaction of integrins with their endothelial ligands (eg ICAM-1) provides the binding force allowing firm adhesion of leucocytes to the endothelial surface under shear stress exerted by the flowing blood. For subsequent crawling of neutrophils along the endothelial layer, integrins have to be continuously shuttled to the leucocytes' leading edge while those at the rear end have to be removed in order to achieve locomotion. As the integrin pool in the cell is limited and crawling is a fast event that cannot be synthesis-dependent, the hypothesis of integrin recycling was put forward in migrating cells, shuffling integrins from the rear to the front.¹⁵ In neutrophils, integrins co-localize with recycling endosomal markers and integrin-containing vesicles are present in both cell front and cell rear. Interestingly, this was shown to be a Ca^{2+} -dependent process since upon Ca^{2+} buffering, vesicles accumulated in the cell rear.¹⁶

Rab11 and its effectors are the major players controlling RE trafficking. Johnson et al showed that Munc13-4, a known effector of Rab27a proposed to support exocytosis through interaction with soluble NSF attachment protein receptors (SNAREs),⁶ additionally binds to Rab11.¹⁷ The same authors also observed an accumulation of Rab11-positive vesicles on the plasma membrane in Munc13-4 deficient neutrophils, suggesting a regulatory role of Munc13-4 in the last steps of membrane fusion (Figure 2A).¹⁷

Two tethering complexes with Rab effector function, class C core vacuole/endosome tethering (CORVET) and homotypic fusion and vacuole protein sorting (HOPS), were also described to be critical for integrin recycling, with CORVET being an effector for Rab5-mediated EE fusion and HOPS an effector for Rab7-dependent fusion of EE to RE.¹⁸ Both complexes were first described in *Caenorhabditis elegans* and are composed of different subunits of the vacuole protein sorting (VPS) family. The mammalian homologues of both proteins share the same core structure, composed of VPS18, VPS16, VPS33A and VPS11. Additionally, CORVET also contains subunits VPS8 and TGFBRAP1, while HOPS contains VPS41 and VPS39. Until recently, little was known about the physiological role of VPS proteins in mammals. Some studies have reported dysfunctional integrin trafficking in migrating cells caused by mutated VPS proteins. In 2012, Peng et al¹⁹ described migration defects in VPS18-deficient murine neurons due to blocking of several vesicle transport pathways to the lysosome. Some years later, VPS3 and VPS8 depletion in HeLa cells was shown to delay the delivery of integrin-containing EE to RE and subsequently impaired integrin recycling leading to defective cell adhesion and spreading, focal adhesion formation and cell migration.²⁰ Concerning neutrophils, a loss of function mutation of VPS45, a protein that interacts with the Rab4/5 effector Rabenosyn-5, was identified to cause a primary immunodeficiency syndrome with severe congenital neutropenia.^{21,22} Neutrophils and fibroblasts isolated from those patients showed reduced mobilization of β_1 integrins to the cell membrane. Moreover, they observed that patients' fibroblasts showed impaired motility and increased apoptosis. Transfection of those cells with functional VPS45 rescued the motility and apoptosis defects confirming that VPS45 is an important regulator of cell motility and cell survival.^{22,23} It would certainly be of great interest to additionally investigate the implications of VPS45 depletion on integrin mobilization and assess its functional significance in neutrophil adhesion to the endothelium and transmigration.

4.2 | Vascular basement membrane penetration

After passing the endothelial layer, neutrophils have to penetrate the vascular basement membrane, an extracellular matrix formation that embeds the endothelial cells and pericytes.

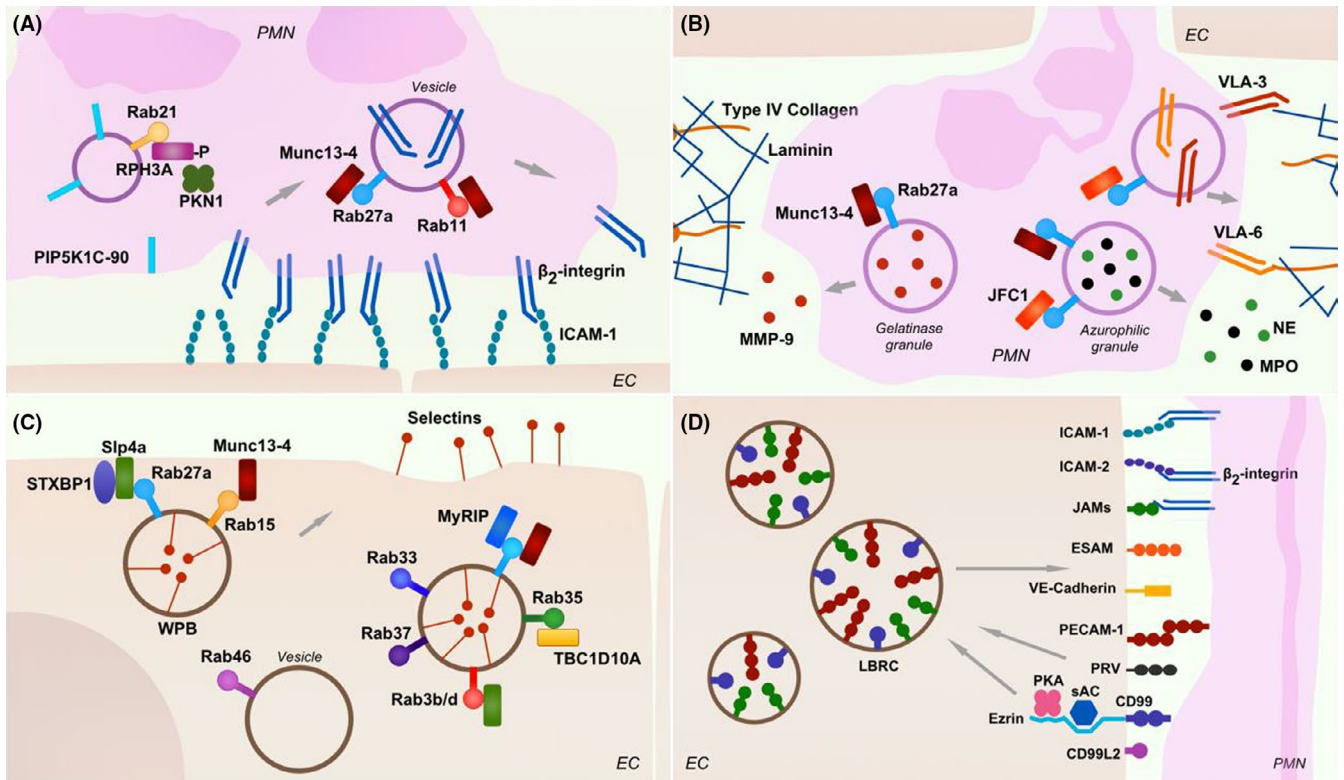


FIGURE 2 Vesicle trafficking during leucocyte recruitment. A, Cell polarization and crawling. PKN1 phosphorylates the Rab21 effector RPH3A and promotes the mobilization of PIP5K1C-90 containing vesicles to the neutrophil plasma membrane, a process required for cell polarization. Rab27a-Munc13-4 and Rab11-Munc13-4 interactions are necessary during the last steps of integrin-containing vesicle fusion to the plasma membrane. B, Vascular basement membrane penetration. Rab27a interacts with Munc13-4 for MMP-9-containing gelatinase granule exocytosis and with Munc13-4 and JFC1 for MPO, and NE-containing azurophilic granules exocytosis. Rab27a-JFC1 is required for the mobilization of vesicles containing VLA-3 and VLA-6. C, Weibel-Palade bodies exocytosis. Rab27a interacting with the effector Slp4a together with Rab15-Munc13-4 interactions promote exocytosis of WPBs. In contrast, Rab27a-Munc13-4 and Rab27a-MyRIP heteromers, as well as Rab3b/d-Slp4a and Rab35-TBC1D10A heteromers co-localize with WPBs and retain them in the periphery. Rab46 drives P-selectin-devoid WPB away from the plasma membrane after stimulation. D, Lateral Border Recycling Compartment (LBRC). LBRC delivers un conjugated PECAM-1, CD99 and JAMs to the EC membrane. PECAM-1 and CD99 activation stimulates the mobilization of the LBRC. CD99 activates PKA via ezrin and sAC. EC; endothelial cell; LBRC, lateral border recycling compartment; PMN, polymorphonuclear cell; WPBs, Weibel-Palade bodies

More than 50 proteins have been identified as components of the vascular basement membrane, such as type IV collagen, laminin, nidogen and perlecan. Both endothelial cells and pericytes can produce basement membrane proteins.

Within all the different vascular basement membrane components, the laminin isoforms are the most important constituents concerning leucocyte trafficking as they define leucocyte extravasation sites. Laminins are composed of 3 subunits (α , β and γ) resulting in 16 possible isoforms. Of those, isoform 411 ($\alpha 4\beta 1\gamma 1$) promotes the transmigration while on the contrary 511 ($\alpha 5\beta 1\gamma 1$) impairs it. Low expression regions (LERs) are areas with a low expression of type IV collagen and laminin 511 that colocalize to cell junctions¹¹ and define preferred neutrophil transmigration sites. Additionally, Song et al in 2017²⁴ demonstrated that adhesion to laminin 511 via β_1 and β_3 integrins affects endothelial barrier function, by stabilizing VE-cadherin and downregulating CD99L2.

In migrating cells, two basement membrane penetration programs have been described: an irreversible, protease dependent programme and a reversible, protease independent programme. The exact mechanism of how neutrophils penetrate the vascular basement membrane is still incompletely understood, but evidence points towards the existence of both penetration programs. On the one hand, neutrophils are continuously trafficking through vascular basement membranes without leaving detectable perforations, unlike in developmental processes or cancer.²⁵ On the other hand, a recent study could observe a NE-induced remodelling of the venular basement membrane after neutrophil infiltration.²⁶ Furthermore, inactivating mutations on metalloproteinase-8 (MMP-8) and didpeptidyl peptidase I (DPPI) in mice showed diminished neutrophil recruitment, even though it did not completely block transmigration.²⁵ However, some basement membranes, such as the one found around bone marrow sinusoids, have been described to be discontinuous and may not require a mechanism of penetration.

In 2014, Singh et al²⁷ found that Rab27b and Rab27a/b double knockout neutrophils showed a transmigration defect in an in vitro transwell migration assay in response to macrophage inflammatory protein 2 (MIP-2) and leukotriene B4 (LTB₄). They observed comparable results during in vivo recruitment in the lung in response to MIP-2. Taking into account that Rab27a and Rab27b play a role in azurophilic granules release⁶ with a high content of proteases, Singh et al suggested that the observed transmigration defect was due to an impaired protease release. Following the same line, Ramadass et al investigated how Rab27a or Munc13-4 deficiency in neutrophils could affect protease secretion and found an impaired release of metalloproteinase-9 (MMP-9) and myeloperoxidase (MPO) (Figure 2B).²⁸ In addition, the same group described a Rab27a-independent mechanism mediated by JFC1 and Ras-related C3 botulinum toxin substrate 1 (Rac1) associated with vesicular trafficking regulation during neutrophil chemotaxis that also affects transmigration patterns.²⁹

A recent publication from our laboratory described impaired recruitment of mammalian STE20-like protein kinase 1 (Mst1)-deficient neutrophils to sites of inflammation in the inflamed murine cremaster muscle. The kinase Mst1 was found to associate with the Rab27a effector JFC1, but not with Munc13-4, and therefore regulating the trafficking of Rab27a-positive vesicles containing integrins VLA-3 and VLA-6, as well as NE.¹² Furthermore, we recently reported that Src family kinase-deficient murine neutrophils apart from showing impaired adhesion also displayed impaired basement membrane penetration. This impairment was due to reduced Rab27a-dependent mobilization of intracellular vesicles containing NE, VLA-3 and VLA-6.³⁰

5 | ENDOTHELIAL VESICULAR TRAFFICKING

5.1 | Weibel-Palade bodies

For proper neutrophil recruitment, correct vesicle trafficking is required in both neutrophils and endothelial cells. Translocation of endothelial P-selectin, contained in WPB, to the endothelial plasma membrane is the first step to initiate the neutrophil recruitment cascade. Rab27a was the first described Rab family member to co-localize with WPB in HUVEC cells. Together with its effector myosin VIIA and Rab interacting protein (MyRIP), it was identified as a negative regulator that anchors mature WPB to peripheral actin filaments, resulting in diminished von Willebrand factor (VWF) release and also WPB exocytosis.³¹ Later on, syntaxin-binding protein 4 (Slp4a), another Rab27a effector, was shown to co-localize with WPB. Interestingly, Slp4a has been demonstrated to function as a positive regulator of

exocytosis³² which was confirmed by Van Breevoort et al³³ showing that the interaction of the Rab27a-Slp4a complex with syntaxin-binding protein 1 (STXB1) was required to promote WPB exocytosis (Figure 2C).

Besides Rab27a several other Rab proteins have been linked to regulate WPB trafficking. Rab3b/d co-localize with WPB and competes with Rab27a for the effector Slp4a. Interestingly, Rab3b/d-Slp4a interaction inhibits VWF secretion.³² In 2012, Zografou et al³⁴ performed a screening of Rab proteins co-localizing with WPBs and found that, apart from Rab27a and Rab3b/d, Rab15, Rab33 and Rab37 interact with WPB. Of those, only Rab15 siRNA inhibition impaired VWF release in cooperation with Rab27a and its effector Munc13-4 (Figure 2C). Recently, GAP activity of overexpressed TBC domain family member 10A (TBC1D10A) on Rab35 was shown to impair WPB exocytosis, while this could be abolished when TBC1D10A-insensitive Rab35 was used. In addition, knockdown of Rab35 inhibited histamine-evoked release of VWF as well as P-selectin.³⁵ This was the first report that directly related the function of a Rab protein to the regulation of selectin surface expression. Thereafter, Rab46 was shown to be involved in the differential regulation of histamine-evoked WPB release by co-localizing with P-selectin-negative WPBs driving away the non-inflammatory cargo from the plasma membrane through retrograde transportation along microtubules.³⁶

5.2 | Lateral border recycling compartment

Endothelial intracellular vesicle transport during transmigration of neutrophils is a prerequisite for neutrophil crossing the endothelial cell layer. Several adhesion-related molecules including ICAM-1, ICAM-2, JAMs, ESAM, PECAM-1, PVR, CD99 and CD99L2 are involved in leucocyte transmigration and act in a sequential manner.^{37,38} All these proteins are enriched at endothelial cell-cell contacts³⁹ making the paracellular transmigration route an ideal location for neutrophil extravasation into inflamed tissue. For initiation of transmigration, Ca²⁺ signalling in endothelial cells plays a major role and is stimulated by PECAM-1 interactions via the ion channel transient receptor potential cation channel subfamily C member 6 (TRPC6).⁴⁰

In 2003, an endothelial vesicle reticulum necessary for leucocyte transmigration was described. This complex was different from vesiculo-vacuolar organelles (VVO), caveolae and endosomes and was located next to the endothelial cell border. It was named lateral border recycling compartment (LBRC) and suggested to provide unligated PECAM-1, CD99 and JAMs but not VE-cadherin to the place of transmigration in a kinesin-mediated, microtubule-dependent manner.⁴¹ Mobilization of LBRC to the cell contacts is initially triggered by PECAM-1 and later on by CD99 through the

activation of protein kinase A (PKA) via ezrin and soluble adenylyl cyclase (sAC).⁴²

Currently, only little is known about the regulation of LBRC by Rab proteins. A characterization study reported that isolated LBRC was largely devoid of Rab5.⁴³ However, Rab5/Rab4 depletion prevented JAM-A redistribution from the interendothelial cell area to the apical surface after inflammatory stimulation⁴⁴ suggesting a role of Rab molecules in the regulation of LBRC.

6 | CONCLUSION

Both, excessive and impaired neutrophil recruitment during the inflammatory response, can be deleterious for the host. Accordingly, neutrophil function, which heavily relies on various proteins that are pre-stored in intracellular vesicles, depends on a tightly regulated machinery for the secretion of its granule content. As described in this review, Rab proteins take an important part in regulating the mobilization of neutrophil recruitment-relevant adhesion and activation proteins that are contained in specific granules/vesicles of neutrophils. Together with different Rab-effector and -adaptor proteins, the function of Rab molecules is highly specific for certain vesicles or steps during vesicle trafficking and even for different cell types. Rab27a, one of the best-studied Rab proteins is of critical importance during neutrophil recruitment for both endothelial cells and neutrophils. Depending on its binding partners, Rab27a is able to promote or inhibit WPB secretion in endothelial cells. In neutrophils, Rab27a guides trafficking of integrin-containing granules during vascular basement membrane penetration and regulates the release of NE or MPO from azurophilic granules during the inflammatory response. Therefore, interfering with these processes through inhibition of Rab molecules would be a promising therapeutic approach. In fact, Nexinhibs (neutrophil exocytosis inhibitors), a new class of drugs, inhibit the binding of Rab27a to its effector JFC1 and are able to decrease azurophilic granule exocytosis in vitro and in vivo. At the same time, nexinhibs leave intact NET formation and phagocytosis capabilities.⁴⁵ Future research is warranted to test these compounds in immune cells. This will potentially open new therapeutic windows in inflammatory diseases with unwanted immune cell recruitment.

ACKNOWLEDGEMENTS

This work was supported by the Else Kröner-Fresenius-Foundation 2015_A68 (IR), Deutsche Forschungsgemeinschaft (DFG) individual grant SP621/5-1 (MS) and Sonderforschungsbereich (SFB) 914 project B01 (MS).

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

ORCID

Markus Sperandio  <https://orcid.org/0000-0002-7689-3613>

REFERENCES

1. Yin C, Heit B. Armed for destruction: formation, function and trafficking of neutrophil granules. *Cell Tissue Res*. 2018;371:455-471.
2. Rørvig S, Honore C, Larsson LI, et al. Ficolin-1 is present in a highly mobilizable subset of human neutrophil granules and associates with the cell surface after stimulation with fMLP. *J Leukoc Biol*. 2009;86:1439-1449.
3. Cowland JB, Borregaard N. Granulopoiesis and granules of human neutrophils. *Immunol Rev*. 2016;273:11-28.
4. Wandinger-Ness A, Zerial M. Rab Proteins and the compartmentalization of the endosomal system. *Cold Spring Harb Perspect Biol*. 2014;6:1-25.
5. Grosshans BL, Ortiz D, Novick P. Rabs and their effectors: achieving specificity in membrane traffic. *Proc Natl Acad Sci*. 2006;103:11821-11827.
6. Johnson JL, Brzezinska AA, Tolmachova T, et al. Rab27a and Rab27b regulate neutrophil azurophilic granule exocytosis and NADPH oxidase activity by independent mechanisms. *Traffic*. 2010;11:533-547.
7. Catz SD. The role of Rab27a in the regulation of neutrophil function. *Cell Microbiol*. 2014;16:1301-1310.
8. Sperandio M, Gleissner CA, Ley K. Glycosylation in immune cell trafficking. *Immunol Rev*. 2009;230:97-113.
9. Schmidt S, Moser M, Sperandio M. The molecular basis of leukocyte recruitment and its deficiencies. *Mol Immunol*. 2013;55:49-58.
10. Vestweber D. How leukocytes cross the vascular endothelium. *Nat Rev Immunol*. 2015;15:692-704.
11. Wang S, Voisin M-B, Larbi KY, et al. Venular basement membranes contain specific matrix protein low expression regions that act as exit points for emigrating neutrophils. *J Exp Med*. 2006;203:1519-1532.
12. Kurz ARM, Pruenster M, Rohwedder I, et al. MST1-dependent vesicle trafficking regulates neutrophil transmigration through the vascular basement membrane. *J Clin Invest*. 2016;126:4125-4139.
13. Xu W, Wang P, Petri B, et al. Integrin-induced PIP5K1C kinase polarization regulates neutrophil polarization, directionality, and in vivo infiltration. *Immunity*. 2010;33:340-350.
14. Yuan Q, Ren C, Xu W, et al. PKN1 directs polarized RAB21 vesicle trafficking via RPH3A and is important for neutrophil adhesion and ischemia-reperfusion injury. *Cell Rep*. 2017;19:2586-2597.
15. Bretscher MS, Aguado-Velasco C. Membrane traffic during cell locomotion. *Curr Opin Cell Biol*. 1998;10:537-541.
16. Paul NR, Jacquemet G, Caswell PT. Endocytic trafficking of integrins in cell migration. *Curr Biol*. 2015;25:R1092-1105.
17. Johnson JL, He J, Ramadass M, et al. Munc13-4 Is a Rab11-binding protein that regulates Rab11-positive vesicle trafficking and docking at the plasma membrane. *J Biol Chem*. 2016;291:3423-3438.
18. Van Der Kant R, Jonker CTH, Wijdeven RH, et al. Characterization of the mammalian CORVET and HOPS complexes and their modular restructuring for endosome specificity. *J Biol Chem*. 2015;290:30280-30290.
19. Peng C, Yan S, Ye J, Shen L, Xu T, Tao W. Vps18 deficiency inhibits dendritogenesis in Purkinje cells by blocking the lysosomal degradation of Lysyl Oxidase. *Biochem Biophys Res Commun*. 2012;423:715-720.

20. Jonker CTH, Galmes R, Veenendaal T, et al. Vps3 and Vps8 control integrin trafficking from early to recycling endosomes and regulate integrin-dependent functions. *Nat Commun*. 2018;9:1-12.
21. Shadur B, Asherie N, Newburger PE, Stepensky P. How we approach: severe congenital neutropenia and myelofibrosis due to mutations in VPS45. *Pediatr Blood Cancer*. 2019;66:e27473.
22. Rahajeng J, Caplan S, Naslavsky N. Common and distinct roles for the binding partners Rabenosyn-5 and Vps45 in the regulation of endocytic trafficking in mammalian cells. *Exp Cell Res*. 2010;316:859-874.
23. Vilboux T, Lev A, Malicdan MCV, et al. A congenital neutrophil defect syndrome associated with mutations in VPS45. *N Engl J Med*. 2013;369:54-65.
24. Song J, Zhang X, Buscher K, et al. Endothelial basement membrane Laminin 511 contributes to endothelial junctional tightness and thereby inhibits leukocyte transmigration. *Cell Rep*. 2017;18:1256-1269.
25. Rowe RG, Weiss SJ. Breaching the basement membrane: who, when and how? *Trends Cell Biol*. 2008;18:560-574.
26. Voisin MB, Nourshargh S. Neutrophil trafficking to lymphoid tissues: physiological and pathological implications. *J Pathol*. 2019;247:662-671.
27. Singh RK, Furze RC, Birrell MA, Rankin SM, Hume AN, Seabra MC. A role for Rab27 in neutrophil chemotaxis and lung recruitment. *BMC Cell Biol*. 2014;15:39.
28. Ramadass M, Johnson JL, Catz SD. Rab27a regulates GM-CSF-dependent priming of neutrophil exocytosis. *J Leukoc Biol*. 2016;101:693-702.
29. Ramadass M, Johnson JL, Marki A, et al. The trafficking protein JFC1 regulates Rac1-GTP localization at the uropod controlling neutrophil chemotaxis and in vivo migration. *J Leukoc Biol*. 2019;105:1209-1224.
30. Rohwedder I, Kurz ARM, Pruenster M, et al. Src family kinase-mediated vesicle trafficking is critical for neutrophil basement membrane penetration. *Haematologica*. 2019;104. <https://doi.org/10.3324/haematol.2019.225722> [Epub ahead of print].
31. Nightingale TD, Pattni K, Hume AN, Seabra MC, Cutler DF. Rab27a and MyRIP regulate the amount and multimeric state of VWF released from endothelial cells. *Blood*. 2009;113:5010-5018.
32. Bierings R, Hellen N, Kiskin N, et al. The interplay between the Rab27A effectors Slp4-a and MyRIP controls hormone-evoked Weibel-Palade body exocytosis. *Blood*. 2012;120:2757-2767.
33. Van Breevoort D, Snijders AP, Hellen N, et al. STXBP1 promotes Weibel-Palade body exocytosis through its interaction with the Rab27A effector Slp4-a. *Blood*. 2014;123:3185-3194.
34. Zografou S, Basagiannis D, Papafotika A, et al. A complete Rab screening reveals novel insights in Weibel-Palade body exocytosis. *J Cell Sci*. 2012;15(125):4780-4790.
35. Biesemann A, Gorontzi A, Barr F, Gerke V. Rab35 protein regulates evoked exocytosis of endothelial Weibel-Palade bodies. *J Biol Chem*. 2017;292:11631-11640.
36. Miteva KT, Pedicini L, Wilson LA, et al. Rab46 integrates Ca²⁺ and histamine signaling to regulate selective cargo release from Weibel-Palade bodies. *J Cell Biol*. 2019;218:2232-2246.
37. Nourshargh S, Hordijk PL, Sixt M. Breaching multiple barriers: leukocyte motility through venular walls and the interstitium. *Nat Rev Mol Cell Biol*. 2010;11:366-378.
38. Filippi MD. Neutrophil transendothelial migration: updates and new perspectives. *Blood*. 2019;133:2149-2158.
39. Goswami D, Vestweber D. How leukocytes trigger opening and sealing of gaps in the endothelial barrier. *F1000Research*. 2016;5:2321.
40. Weber EW, Han F, Tauseef M, Birnbaumer L, Mehta D, Muller WA. TRPC6 is the endothelial calcium channel that regulates leukocyte transendothelial migration during the inflammatory response. *J Exp Med*. 2015;212:1883-1899.
41. Mamdouh Z, Mikhailov A, Muller WA. Transcellular migration of leukocytes is mediated by the endothelial lateral border recycling compartment. *J Exp Med*. 2009;206:2795-2808.
42. Watson RL, Buck J, Levin LR, et al. Endothelial CD99 signals through soluble adenylyl cyclase and PKA to regulate leukocyte transendothelial migration. *J Exp Med*. 2015;212:1021-1041.
43. Sullivan DP, Rüffer C, Muller WA. Isolation of the lateral border recycling compartment using a diaminobenzidine-induced density shift. *Traffic*. 2014;15:1016-1029.
44. Stamatovic SM, Sladojevic N, Keep RF, Andjelkovic AV. Relocalization of junctional adhesion molecule a during inflammatory stimulation of brain endothelial cells. *Mol Cell Biol*. 2012;32:3414-3427.
45. Johnson JL, Ramadass M, He J, et al. Identification of neutrophil exocytosis inhibitors (Nexinhibs), small molecule inhibitors of neutrophil exocytosis and inflammation: druggability of the small GTPase Rab27a. *J Biol Chem*. 2016;291:25965-25982.

How to cite this article: Masgrau-Alsina S, Sperandio M, Rohwedder I. Neutrophil recruitment and intracellular vesicle transport: A short overview. *Eur J Clin Invest*. 2020;50:e13237. <https://doi.org/10.1111/eci.13237>